

Review Article

Role of Regulatory T Cells in Tumor-Bearing Mice Treated with Allo-Hematopoietic Stem Cell Transplantation Plus Thymus Transplantation

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We recently developed a new allogeneic hematopoietic stem cell transplantation method (allo-HSCT) combined with thymus transplantation (TT) from the same donor (allo-HSCT + TT). This method induces elevated T cell function with mild graft-versus-host disease (GVHD) in comparison to conventional HSCT alone and HSCT + donor lymphocyte infusion (DLI). This new method is effective against several intractable diseases, including malignant tumors, for which conventional treatments are ineffective. Regulatory T (T_{reg}) cells play an important role in the enhanced graft-versus-tumor (GVT) effect and reduction of GVHD, thus leading to longer survival. Replacement and reduction of elevated T_{reg} cells by donor-derived allo- T_{reg} cells from the transplanted thymus may play one of crucial roles in the effect. This review discusses the role of T_{reg} cells in a tumor-bearing mouse model treated with allo-HSCT + TT.

1. Introduction

We recently developed a new allogeneic hematopoietic stem cell transplantation method (allo-HSCT) in conjunction with thymus transplantation (TT) from the same donor (allo-HSCT + TT) [1–11]. This method results in elevated T cell function with mild graft-versus-host disease (GVHD) compared to HSCT alone or HSCT + donor lymphocyte infusion (HSCT + DLI) [8]. The mechanism underlying these effects involves $CD4^+$ FoxP3⁺ regulatory T (T_{reg}) cells, which suppress immune activity and prevent autoimmunity and GVHD [12, 13]. The percentages of these cells in $CD4^+$ T cells are intermediate between HSCT alone and HSCT + DLI, while the opposite is true for the percentage of $CD4^+$ FoxP3⁻ effector T (T_{eff}) cells. There are two main ways of producing T_{reg} cells—that is, from the thymus (as naturally occurring T_{reg} , nT_{reg}) and from peripheral cells (inducible T_{reg} , iT_{reg}) [14, 15]. We observed that not only the number of T cells but also the quantity of T cell receptor rearrangement excision circles (TREC) [8], which reflect production of T cells from

the thymus, are increased in HSCT + TT. Although we did not purify the T_{eff} and T_{reg} cells in TREC analysis, we suggest that both naive cells are produced from the transplanted thymus and move to the periphery because of fundamentally similar mechanisms of them for those cells [16].

This method showed efficacy against several intractable diseases and conditions, such as autoimmune diseases in aging and radioresistant hosts [2, 3], exposure to supralethal irradiation [4], multiple-organ transplantation from different donors [5], type 2 diabetes mellitus [6], low hematopoietic stem cell (HSC) number or low dose of irradiation [7], and malignant tumors, including leukemia [8–11]. Malignant tumor-bearing mice treated with allo-HSCT + TT showed a strong graft-versus-tumor (GVT) effect but weak GVHD compared with HSCT alone and HSCT + DLI. These effects may involve replacement and reduction of the elevated T_{reg} cells by allo- T_{reg} cells.

The regulation of T_{reg} cells was suggested to be one mechanism of action of immunotherapy for cancer, and this has

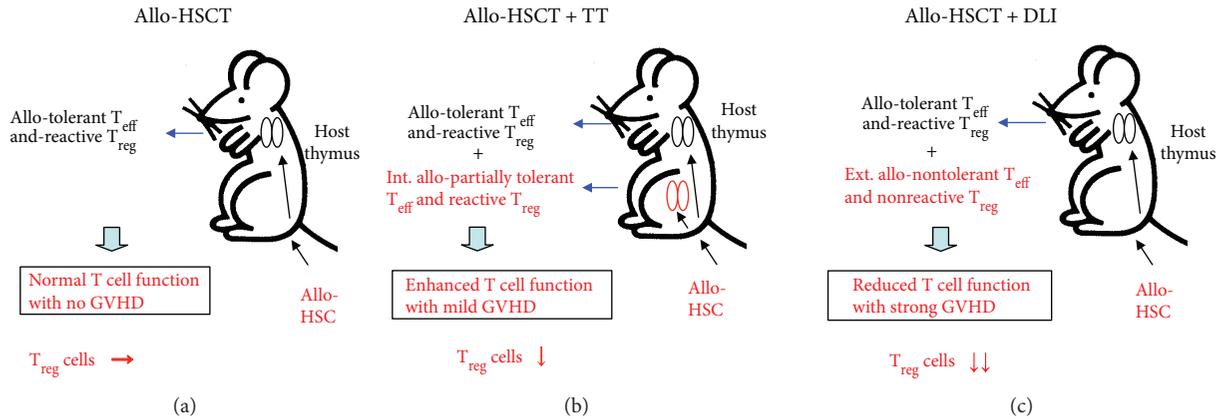


FIGURE 1: Theory of allo-HSCT + TT. In the case of conventional allo-HSCT (a), allo- T_{eff} and T_{reg} cells develop, are tolerated, and react in the host thymus. No GVHD occurs. The proportion of T_{reg} cells is comparable to that in normal mice. In the case of allo-HSCT + DLI (c), allo-nontolerant T_{eff} and nonreactive T_{reg} cells are externally supplied, and strong GVHD is induced with reduction of T cell function. The proportion of T_{reg} cells is markedly reduced. In the case of allo-HSCT + TT, the allo- T_{eff} and T_{reg} cells develop internally in the allo-transplanted thymus. The T cells show partial tolerance and reaction with the host, and only mild GVHD occurs with elevation of T cell function (b). The proportion of T_{reg} cells decreases slightly. Figure 1 is modified from Hosaka [1], under the Creative Commons Attribution License/public domain.

been examined in clinical trials [17]. It may also be applicable under allo-HSCT + TT. We review and discuss the utility of T_{reg} cells for treatment of cancer.

2. Main Text

2.1. Review

2.1.1. Theory of HSCT + TT with T_{reg} Cells. First, we present the theory of allo-HSCT + TT [1, 8]. This method makes use of intra-bone marrow-bone marrow transplantation (IBM-BMT) for HSCT, which involves the direct injection of HSC into the bone marrow cavity, and results in superior engraftment of donor cells and reduced incidence of GVHD with mesenchymal stem cells (MSC) [18–20]. In the case of conventional allo-HSCT, allo-HSC are transplanted into the host, and allo-T cells develop in the host thymus (Figure 1(a)). The T_{eff} cells induce tolerance toward the host with thymic antigen-presenting cells (APC) and/or epithelial cells (TEC) [21]. Host-reactive T_{reg} cells are also reacted with host thymic dendritic cells (DC) [22]. Neither T cell type induces apparent GVHD, and the proportion of T_{reg} cells is comparable to that in normal mice. In contrast, nontolerant allo- T_{eff} and nonreactive T_{reg} cells are externally supplied in the case of HSCT + DLI, resulting in strong GVHD (Figure 1(c)). As this results in expansion of T_{eff} cells and little proliferation of T_{reg} cells, the proportion of T_{reg} cells is markedly reduced. In HSCT + TT (Figure 1(b)), allo- T_{eff} and T_{reg} cells develop internally from the transplanted allo-thymus in the host. The T_{eff} and T_{reg} cells are partially tolerant and reactive to the host, which was suggested to show a low response in mixed lymphocyte reaction, resulting in low GVHD [8]. Under these conditions, most allo- T_{eff} cells derived from the transplanted thymus are in the naïve state and may not expand well to host antigens. The T_{reg} cells also suppress activation of naïve cells by deprivation of activation

signals [23]. Therefore, T_{reg} cells may play a role in allo-HSCT + TT. Nonetheless, the degree of inhibition may be insufficient, leading to mild GVHD with a slight decrease in the proportion of T_{reg} cells.

2.1.2. Effects of Allo-HSCT + TT in Tumor-Bearing Mice and the Dynamics of T_{reg} Cells. Next, we describe the effects of HSCT + TT in tumor-bearing mice and the dynamics of T_{reg} cells (Table 1). Non-tumor-bearing mice without treatment, or those treated with HSCT alone, with HSCT + adult thymus (AT) transplantation, or with HSCT + DLI, were used as representative non-tumor-bearing controls (Figure 1, group 1). The tumor-bearing mice showed an increase in T_{reg} cell number with inducible T_{reg} cells [24]. Treatment with HSCT in the early phase of tumor progression (group 2a) resulted in a reduction in the proportion of T_{reg} cells among $CD4^+$ T cells, although they were still elevated compared with non-tumor-bearing mice. In HSCT + AT treatment, the proportion of T_{reg} cells decreased further and was comparable to the level in non-tumor-bearing mice. The mice showed the longest survival with strong GVT effects and mild GVH effects.

The functions of the transplanted thymus from AT, newborn thymus (NT), and fetal thymus (FT) in mice treated with HSCT were compared (group 2b, c), as the functions differ between ages. The proportion of T_{reg} cells did not change with any type of HSCT alone or HSCT + TT, and all of the HSCT + TT mice showed strong GVT and longer survival compared to nontreated controls or those treated with HSCT alone. However, the GVT effects in HSCT + NT or FT transplantation were greater than those of HSCT + AT transplantation, and the survival was longest in HSCT + NT transplantation. These animals showed the highest levels of $IFN\gamma$ and effector memory (EM) T cells and the lowest numbers of myeloid suppressor cells [10].

TABLE 1: Effects of T_{reg} cells in tumor-bearing mice treated with allo-HSCT + TT

Group	TT	No treated [#]	Comparison of T_{reg} cells*			Effect of HSCT + TT [§]	Ref.
			HSCT	HSCT + TT	HSCT + DLI		
1. No tumor	AT		→	↓	↓↓	aGVH, elevated T cell function	[1, 8]
2a. Early tumor	AT	↑↑	↑	→	ND	GVT ^a , LS ^a ,	[8, 10]
2b. Same as above	NT	↑↑	↑	→	ND	GVT ^b , LS ^b , IFN γ ^b , EM T cells ^b , and MS cells ^c	[10]
2c. Same as above	FT	↑↑	↑	→	ND	GVT ^b , LS ^d	[10]
3. Advanced tumor	FT	↑↑↑	↑↑	↑	ND	Inhibition of metastasis, LS	[9]
4. Leukemia	AT	ND	→	↓	↓↓	GVL, aGVH, and LS	[11]

*% of FoxP3⁺ cells in CD4⁺ T cells compared with non-tumor-bearing mice: no change, →; mild increase, ↑; moderate increase, ↑↑; strong increase, ↑↑↑; slight decrease, ↓; moderate decrease, ↓↓. [#]Host-derived cells. [§]Compared with HSCT and/or HSCT + DLI in the same group. ^a3rd in group 2, ^b1st in group 2, ^clowest in group 2, ^d2nd in group 2. AT: adult thymus; NT: newborn thymus; FT: fetal thymus; aGVH: attenuated graft-versus-host; GVT: graft-versus-tumor; LS: longest survival; EM: effector memory; MS: myeloid suppressor; GVL: graft-versus-leukemia; ND: not determined.

In advanced tumors (group 3), nontreated tumor-bearing mice showed marked elevation of T_{reg} cell number. HSCT + TT reduced the T_{reg} cell number to a greater extent than did HSCT alone and inhibited lung metastasis leading to the longest survival, although the T_{reg} cell level did not decrease to normal and there was no significant regression of the primary tumor [9].

The results with regard to T_{reg} cells in leukemia-bearing mice in group 4 were similar to those of non-tumor-bearing mice in group 1. The T_{reg} cell number in the HSCT + AT transplantation group was intermediate between those of HSCT alone and HSCT + DLI, and HSCT + AT transplantation yielded the longest survival with the greatest graft-versus-leukemia (GVL) effect and attenuated GVHD [11].

2.2. Discussion. HSCT + TT is a valuable method for treatment of cancer, and T_{reg} cells play a crucial role in mediating the effects of this method. As shown in Figure 2, T_{reg} cell number was elevated in untreated hosts bearing tumors and increased with tumor progression (Figure 2(a)). Tumor cells produce TGF β , which induces iT $_{reg}$ cells leading to inhibition of immune reaction against cancer [25, 26]. Allo-HSCT alone showed a mild GVT effect by allo-reaction with a slight reduction in T_{reg} cell number compared to syngeneic HSCT [9] (Figure 2(b)). Additional transplantation of thymus grafts showed a further GVT effect with further reduction in T_{reg} cell number (Figure 2(c)). The level of GVT was comparable to that from HSCT + DLI leading to long survival, although animals treated with HSCT + DLI showed higher GVHD and shorter survival with lower T_{reg} cell number (Figure 2(d)). These findings were consistent with an important role of T_{reg} cells in inducing strong GVT effects and mild GVH effects in HSCT + TT [27].

Thymic function is known to differ according to age [28–30]. Therefore, we next performed comparisons between fetal, newborn, and adult thymic grafts. Although the proportion of T_{reg} cells was the same in all of these groups, NT showed the best effect with regard to GVT and survival. This may have been related to its strong reduction of myeloid suppressor cells, which inhibit immune activity [31, 32], and elevated production of effector memory T cells and IFN γ [10]. Although the detailed mechanism is not yet

clear, it is possible that NT shows the highest function of T cell production among the thymus grafts [4].

Mice bearing advanced tumors showed further elevation of T_{reg} cell level. Therefore, the level was not normalized by HSCT + TT, and the primary tumor did not show significant regression. Nonetheless, they showed inhibition of metastasis and long-term survival, suggesting that this method is still effective with regard to GVT on newly developed tumor cells.

Although mice bearing leukemia showed similar results, those treated with either HSCT + TT or DLI showed long survival with complete remission of tumor cells by donor-derived cells. Therefore, the latter may have reduced production of T_{reg} cells from the tumor and/or thymus graft leading to greater GVHD than the former.

Some of our data were based on conversion of T_{eff} cells to T_{reg} cells in the tumor microenvironment [24, 25]. However, a recent study involving analysis of TCR repertoires in a mouse model using chemical carcinogen-induced fibrosarcoma showed that such conversion does not occur [33]. Although the reason for the discrepancy remains unclear, it is possible that the properties of the tumors were different between the studies. Generally, cancers develop with gradual accumulation of gene mutations and express cancer antigens accompanied by immune reactions involving T_{eff}/T_{reg} interaction and/or conversion. The specific carcinogen-induced tumor is unknown to be the same condition. Further studies are required using several cancer models developed with different mechanisms.

The mechanism underlying the production of T_{reg}/T_{eff} cells from allo-TT has not been clarified under cancer-bearing conditions. As shown in Figure 3, the host antigen comes into direct contact with the transplanted thymus from renal capsules, in which it is translated, whereas cancer antigens are relatively isolated from and do not come into direct contact with the transplanted thymus, as tumor cells were transplanted subcutaneously into the backs of the experimental animals [8–10]. This may lead to host antigen-specific T_{reg} cells being superior to cancer antigens for inducing thymic DC in the transplanted thymus, likewise tolerance with intrathymical administration [34], which may result in inhibition of GVHD, but not GVT.

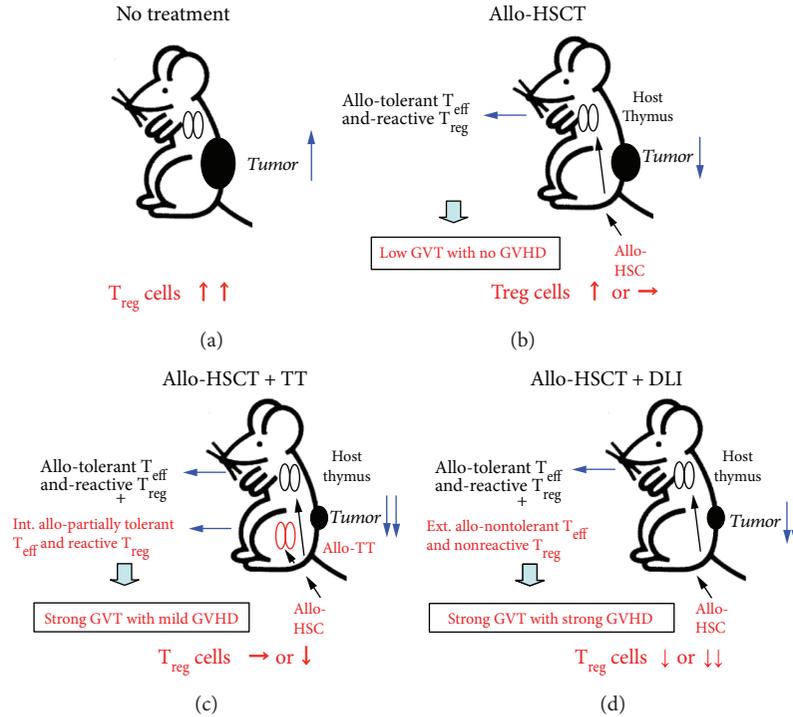


FIGURE 2: Theory of allo-HSCT + TT for tumors. Untreated controls bearing tumor tissue showed increased numbers of T_{reg} cells, including iT_{reg} cells, with tumor progression (a). In the case of conventional allo-HSCT (b), the allo- T_{eff} and T_{reg} cells develop with tolerance and reactivity to the host in the thymus. A low GVT effect is then induced with no/minimal GVHD. Mild tumor regression is induced compared with untreated controls (a). The proportion of T_{reg} cells still increases slightly or is at the normal level. In the case of allo-HSCT + DLI (d), nontolerant and nonreactive allo- T_{eff} and T_{reg} cells are supplied externally, and a strong GVT effect occurs with strong GVHD. The proportion of T_{reg} cells decreases either slightly or markedly. In the case of allo-HSCT + TT (c), internally allo-partially tolerant T_{eff} and reactive T_{reg} are induced from the transplanted thymus. As a result, strong GVT occurs with mild GVHD. The proportion of T_{reg} cells decreases slightly or is at the normal level.

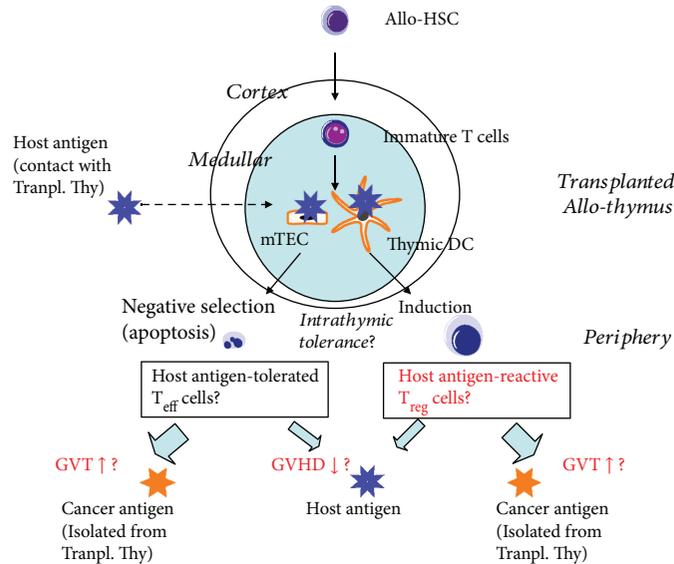


FIGURE 3: Hypothesis for immune regulation of malignant tumor by allo-HSCT + TT. The host antigen comes into direct contact with the transplanted thymus, whereas cancer antigens are relatively isolated from the transplanted thymus. Therefore, host antigen-specific T_{reg} cells may be induced intrathymically to a greater extent than those specific to cancer antigens with thymic DC. Conversely, T_{eff} cells are tolerant toward host antigens, but not cancer antigens with medullary TEC (mTEC), yielding the same results. This may result in strong GVT with mild GVHD. Figure 3 is modified from Hosaka [1], under the Creative Commons Attribution License/public domain.

Conversely, T_{eff} cells are tolerant toward host antigens, but not cancer antigens, yielding the same results.

The regulation of T_{reg} cells is regarded as a suitable target for immune therapy in human cancers and has been the subject of several clinical trials. The main purpose is effective deletion of T_{reg} cells to enhance immune function against tumor cells [35]. Treatment with antibodies for cytotoxic T lymphocyte-associated antigen (CTLA) 4, which is expressed constitutively in T_{reg} cells, or CC chemokine receptor (CCR) 4, which is expressed in activated T_{reg} cells, has been shown to have beneficial effects against melanoma, renal cell carcinoma, and bladder cancer [36–39]. Administration of metronomic cyclophosphamide, which reduces highly proliferative T_{reg} cells, has beneficial effects on advanced cancers and metastatic breast cancers [40, 41]. As a side effect, blockade of immune checkpoints, such as CTLA-4 and programmed death (PD) 1, may induce serous autoimmune diseases [42–44]. In such cases, targeted depletion of tumor-infiltrating T_{reg} cells has been suggested [45]. In addition, it is also important to assess susceptibility to autoimmune diseases in patients with human lymphocyte antigen (HLA) haplotype and monitoring the number of T_{reg} cells.

Although allo-HSCT + TT shows the opposite immune reaction to these reports, the basic theory and points of note are similar. Although allo-T cells can induce GVT, the method using the above antibodies may be applicable in cases when the immune reaction is insufficient. In contrast, if the reaction is too strong with GVHD as autoimmune disease, iT_{reg} can be induced in vivo and/or ex vivo by treatment with IL-10 and/or TGF β and subsequently transferred to the host [46, 47].

Taken together, allo-HSCT + TT is effective for treatment of malignant tumors, and T_{reg} cells may play one of crucial roles in the regulation. Among TT from various ages, NT showed the best functionality. Therefore, regenerative thymus tissue would be better than surgically obtained tissue. With recent progress in engineering for thymus regeneration [48–52], HSCT + TT may be useful as next-generation therapy for treatment of human cancer with control of T_{reg} cells.

3. Conclusions

T_{reg} cells play a crucial role in allo-HSCT + TT for treatment of malignant tumors. Additional control and regulation of T_{reg} cells may lead to better results, and this method may be applicable to human cancer.

Abbreviations

allo-HSCT:	Allogeneic hematopoietic stem cell transplantation
TT:	Thymus transplantation
AT:	Adult thymus
DLI:	Donor lymphocyte infusion
FT:	Fetal thymus
NT:	Newborn thymus
GVHD:	Graft-versus-host disease
GVT:	Graft versus tumor

GVL:	Graft-versus-leukemia
HSCT:	Hematopoietic stem cell transplantation
T_{eff} :	Effector T cell
T_{reg} :	Regulatory T cell
nT_{reg} :	Naturally occurring T_{reg}
iT_{reg} :	Inducible T_{reg}
DC:	Dendritic cell
TEC:	Thymic epithelial cell.

Conflicts of Interest

The author declares that there are no conflicts of interest regarding the publication of this article.

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